In the Claims

Please amend the claims as follows:

1. (Original) A method to detect the presence or amount of a first molecule for a first enzyme-mediated reaction and a second molecule for a second enzyme-mediated reaction, comprising:

a) contacting a sample with a reaction mixture for the first reaction and for the second reaction, wherein a reaction mediated by the first enzyme yields a luminogenic product, and wherein a reaction mediated by the second enzyme yields a nonluminogenic product; and

b) detecting the presence or amount of the first and the second molecules in the sample.

2. (Original) The method of claim 1 wherein the first molecule is a substrate for the first enzyme-mediated reaction.

3. (Original) The method of claim 1 wherein the second molecule is a substrate for the second enzyme-mediated reaction.

4. (Original) The method of claim 1 wherein the first molecule is an enzyme for the first enzyme-mediated reaction.

5. (Original) The method of claim 1 wherein the second molecule is an enzyme for the second enzyme-mediated reaction.

6. (Original) The method of claim 1 wherein the first molecule is a co-factor for the first enzyme-mediated reaction.

7. (Original) The method of claim 1 wherein the second molecule is a co-factor for the second enzyme-mediated reaction.

8. (Original) The method of claim 1 wherein luminescence is employed to detect the first molecule.

9. (Original) The method of claim 1 wherein fluorescence is employed to detect the second molecule.

10. (Original) The method of claim 1 wherein the presence or amount of the first and second molecules is detected sequentially.

11. (Original) The method of claim 1 wherein the sample is a cell lysate.

12. (Original) The method of claim 1 wherein the sample is contacted with the reaction mixture for the first reaction before the reaction mixture for the second reaction.

13. (Original) The method of claim 1 wherein the sample is contacted with the reaction mixture for the second reaction before the reaction mixture for the first reaction.

14. (Original) The method of claim 1 wherein the sample is contacted with the reaction mixture for the first reaction and the second reaction at the same time.

15. (Currently Amended) A method to detect the presence or amount of a first enzyme or cofactor for a first enzyme-mediated reaction, comprising:
a) contacting a sample with a first substrate for the first enzyme, a second substrate for a second enzyme, and optionally a third enzyme, wherein a reaction between the first substrate and the first enzyme or a reaction between the third enzyme and a product of a reaction between the first enzyme and the first substrate yields a luminogenic product,

wherein the second substrate and/or a product of a reaction between the second substrate and the second enzyme is/are not luminogenic; and

b) detecting the presence or amount of the first enzyme or cofactor for the reaction mediated by the first enzyme.

16. (Currently Amended) A method of assaying an enzyme-mediated luminescence reaction to detect the presence or amount of an enzyme or cofactor for a first enzyme-mediated reaction, comprising:

a) contacting a sample with a first substrate for a first enzyme, a second substrate for a second enzyme, and optionally a third enzyme, wherein a reaction between the first substrate and the first enzyme or a reaction between the third enzyme and a product of the reaction between the first enzyme and the first substrate yields a luminogenic product, wherein the second substrate and/or a product of a reaction between the second substrate and the second enzyme is/are not luminogenic; and

b) detecting luminescence.

17. (Original) The method of claim 15 wherein luminescence is detected.

18. (Original) The method of claim 15 or 16 wherein luminescence increases in the presence of the first enzyme or cofactor.

19. (Original) The method of claim 15 or 16 wherein luminescence decreases in the presence of the first enzyme or cofactor.

20. (Original) The method of claim 15 or 16 further comprising detecting the presence or amount of the second enzyme.

21. (Original) The method of claim 20 wherein fluorescence is employed to detect the presence or amount of the second enzyme.

22. (Original) The method of claim 20 wherein the presence or amount of the first enzyme or cofactor and the presence or amount of the second enzyme are detected sequentially.

23. (Original) The method of claim 20 wherein the presence or amount of the first enzyme or cofactor and the presence or amount of the second enzyme are detected simultaneously.

24. (Original) The method of claim 20 wherein the presence or amount of the second enzyme is detected colorimetrically.

25. (Original) The method of claim 20 wherein the presence or amount of the second enzyme is detected by contacting the sample with a fourth enzyme and third substrate for a reaction between the product of the reaction between the second substrate and second enzyme which yields a fluorogenic product.

26. (Original) The method of claim 15 or 16 further comprising detecting the presence or amount of the nonluminogenic second substrate or the nonluminogenic product of the reaction between the second substrate and the second enzyme.

27. (Original) The method of claim 15 or 16 wherein the second enzyme does not react substantially with the first substrate.

28. (Original) The method of claim 15 or 16 wherein the first enzyme does not react substantially with the second substrate.

29. (Original) The method of claim 15 or 16 wherein the second substrate or the product of the reaction between the second substrate and the second enzyme is fluorescent.

30. (Currently Amended) The method of claim 29 wherein the second substrate or the product of the reaction between the second substrate and the second enzyme comprises ethidium bromide, fluorescein, Cy3, BODIPY, a rhodol, Rox, 5-carboxyfluorescein, 6-carboxyfluorescein, an anthracene, 2-amino-4-methoxynapthalene, a phenalenone, an acridone, fluorinated xanthene derivatives, α-naphtol, β-napthol, 1-hydroxypyrene, coumarin, 7-amino-4-methylcoumarin (AMC), 7-amino-4-trifluoromethylcoumarin (AFC), Texas Red, tetramethylrhodamine, carboxyrhodamine, ~~or~~ rhodamine, cresyl, rhodamine-110 or resorufin.

31. (Original) The method of claim 15 or 16 wherein one enzyme is a glycosidase, phosphatase, kinase, dehydrogenase, peroxidase, sulfatase, peptidase, or hydrolase.

32. (Original) The method of claim 15 or 16 wherein one enzyme is a protease.

33. (Original) The method of claim 32 wherein one enzyme is a caspase.

34. (Original) The method of claim 33 wherein the caspase includes caspase-3, caspase-7 or caspase-8.

35. (Original) The method of claim 15 or 16 wherein one of the substrates comprises DEVD, WEHD, LEHD, VEID, VEVD, VEHD, IETD, AEVD, LEXD, VEXD, IEHD, PEHD, ZEVD or LETD.

36. (Original) The method of claim 15 or 16 wherein one of the substrates comprises X_1-X_2-X_3-D wherein X_1 is Z, Y, D, L, V, I, A, W or P, X_2 is V or E, and X_3 is any amino acid.

37. (Original) The method of claim 15 or 16 wherein one of the substrates is a substrate for trypsin or tryptase.

38. (Original) The method of claim 15 or 16 wherein one enzyme cleaves a substrate comprising arginine or lysine.

39. (Original) The method of claim 15 or 16 wherein the sample is a cell lysate.

40. (Original) The method of claim 39 wherein the sample is a cellular sample that is treated with a cell death inducing agent prior to lysis.

41. (Original) The method of claim 15 or 16 wherein the sample comprises intact cells.

42. (Original) The method of claim 15 or 16 wherein the third enzyme is a luciferase.

43. (Original) The method of claim 42 wherein the luciferase is a beetle luciferase.

44. (Original) The method of claim 15 or 16 wherein the second substrate is a substrate for lactate dehydrogenase.

45. (Original) The method of claim 15 or 16 wherein the sample is contacted with the first substrate before the second substrate.

46. (Original) The method of claim 15 or 16 wherein the sample is simultaneously contacted with the first and the second substrates.

47. (Original) The method of claim 15 or 16 wherein the sample is contacted with the second substrate before the first substrate.

48. (Original) The method of claim 15 or 16 wherein the presence or amount of the cofactor is detected by contacting the sample with the first enzyme.

49. (Currently Amended) A method to detect the presence or amount of at least two molecules in a sample, comprising:

a) contacting the sample with a first substrate for a first enzyme, a second substrate for a second enzyme and optionally a third enzyme, wherein a reaction between the first substrate and the first enzyme or the third enzyme and a product of a reaction between the first enzyme and first substrate yields a luminogenic product, wherein the second substrate and/or a product of a reaction between the second substrate and the second enzyme ~~are~~ is/are not luminogenic, and wherein the first and second enzymes are not the same; and

b) detecting the presence or amount of the first and second enzymes or a cofactor for a reaction mediated by the first or second enzyme.

50. (Original) The method of claim 49 wherein luminescence is detected.

51. (Original) The method of claim 49 wherein at least one enzyme is a protease.

52. (Original) The method of claim 49 wherein the second enzyme does not react substantially with the first substrate.

53. (Original) The method of claim 49 wherein the first enzyme does not react substantially with the second substrate.

54. (Original) The method of claim 49 wherein the second substrate or the product of the reaction between second substrate and the second enzyme is fluorescent.

55. (Original) The method of claim 49 wherein fluorescence is employed to detect the presence or amount of the second enzyme or cofactor.

56. (Original) The method of claim 49 wherein at least one enzyme is a caspase.

57. (Original) The method of claim 49 wherein one of the substrates is a substrate for trypsin or tryptase.

58. (Original) The method of claim 49 wherein the sample is a cell lysate.

59. (Original) The method of claim 58 wherein the sample is a cellular sample that is treated with a cell death inducing agent prior to lysis.

60. (Original) The method of claim 49 wherein the sample comprises intact cells.

61. (Original) The method of claim 49 wherein the third enzyme is a luciferase.

62. (Original) The method of claim 61 wherein the luciferase is a beetle luciferase.

63. (Original) The method of claim 62 wherein the second substrate or the product of the reaction between second substrate and the second enzyme is fluorescent.

64. (Original) The method of claim 49 wherein the sample is contacted with the first substrate before the second substrate.

65. (Original) The method of claim 49 wherein the sample is contacted with the second substrate before the first substrate.

66. (Original) The method of claim 49 wherein the sample is simultaneously contacted with the first and the second substrates.

67. (Original) The method of claim 49 wherein the presence or amount of the first enzyme or first cofactor is detected before the presence or amount of the second enzyme or second cofactor is detected.

68. (Original) The method of claim 49 wherein the presence or amount of the second enzyme or second cofactor is detected before the presence or amount of the first enzyme or first cofactor is detected.

69. (Original) The method of claim 68 wherein the second substrate or the product of the reaction between second substrate and the second enzyme is fluorescent.

70. (Currently Amended) The method of claim 69 wherein the second substrate or the product of the reaction between the second substrate and the second enzyme comprises ethidium bromide, fluorescein, Cy3, BODIPY, a rhodol, Rox, 5-carboxyfluorescein, 6-carboxyfluorescein, an anthracene, 2-amino-4-methoxynapthalene, a phenalenone, an acridone, fluorinated xanthene derivatives, α-naphtol, β-napthol, 1-hydroxypyrene, coumarin, 7-amino-4-methylcoumarin (AMC), 7-amino-4-trifluoromethylcoumarin (AFC), Texas Red, tetramethylrhodamine, carboxyrhodamine, ~~or~~ rhodamine, cresyl, rhodamine-110 or resorufin.

71. (Original) The method of claim 15, 16 or 49 wherein the cofactor is ATP.

72. (Original) The method of claim 49 wherein the presence or amount of the second enzyme is detected by contacting the sample with a fourth enzyme and third substrate for a reaction between the product of the reaction between the second substrate and second enzyme which yields a fluorogenic product.

73. (Original) A method to detect the presence or amount of a molecule for a first enzyme-mediated reaction, comprising:
a) contacting a sample which comprises cells which express a fluorescent protein with a reaction mixture for the first enzyme-mediated reaction, wherein a reaction mediated by the first enzyme yields a luminogenic product; and
b) detecting the presence or amount of the molecule and the presence or amount of the fluorescent protein in the sample.

74. (Original) The method of claim 73 wherein the molecule is a substrate.

75. (Original) The method of claim 73 wherein the molecule is a co-factor.

76. (Original) The method of claim 73 wherein the molecule is an enzyme.

77. (Original) A kit comprising:
a nonluminogenic substrate; and
a luminogenic substrate.

78. (Original) The kit of claim 77 further comprising an enzyme capable of mediating a luminescence reaction with the luminogenic substrate.

79. (Original) The kit of claim 77 further comprising instructions for conducting a luminogenic reaction and a nonluminogenic reaction in a single reaction vessel which comprises the nonluminogenic substrate and the
luminogenic substrate.

80. (Original) A kit comprising:
a nonluminogenic substrate; and
an enzyme capable of mediating a luminescence reaction.

81. (Original) The kit of claim 80 further comprising a luminogenic substrate for the enzyme.

82. (Original) The kit of claim 77 or 80 wherein the nonluminogenic substrate is a fluorogenic substrate.

83. (Original) The kit of claim 78 or 80 wherein the enzyme is a luciferase.

84. (Original) The kit of claim 80 further comprising instructions for conducting a luminogenic reaction and a nonluminogenic reaction in a single reaction vessel which comprises the nonluminogenic substrate and the enzyme.